Developmental Biology **218,** 53–63 (2000)
doi:10.1006/dbio.1999.9570, available online at http://www.idealibrary.com on IDEAL®

Cyclin A Associates with the Fusome during Germline Cyst Formation in the *Drosophila* Ovary

Mary A. Lilly,*,† Margaret de Cuevas,*,[1] and Allan C. Spradling*

**Department of Embryology/Howard Hughes Medical Institute, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, Maryland 21210; and †Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892*

Regulated changes in the cell cycle underlie many aspects of growth and differentiation. Prior to meiosis, germ cell cycles in many organisms become accelerated, synchronized, and modified to lack cytokinesis. These changes cause cysts of interconnected germ cells to form that typically contain 2^n cells. In *Drosophila,* developing germ cells during this period contain a distinctive organelle, the fusome, that is required for normal cyst formation. We find that the cell cycle regulator Cyclin A transiently associates with the fusome during the cystocyte cell cycles, suggesting that fusome-associated Cyclin A drives the interconnected cells within each cyst synchronously into mitosis. In the presence of a normal fusome, overexpression of Cyclin A forces cysts through an extra round of cell division to produce cysts with 32 germline cells. Female sterile mutations in *UbcD1,* encoding an E2 ubiquitin-conjugating enzyme, have a similar effect. Our observations suggest that programmed changes in the expression and cytoplasmic localization of key cell cycle regulatory proteins control germline cyst production.

Key Words: **Cyclin A; cell cycle; fusome; oogenesis; *Drosophila.***

INTRODUCTION

Developmentally regulated changes in the cell cycle play important roles at several early steps in *Drosophila* oogenesis. The onset of meiosis and oocyte differentiation is preceded by a series of unusual mitotic divisions that generate small groups of interconnected germline cells known as cysts (Telfer, 1975; de Cuevas *et al.,* 1997). Cyst development begins with the asymmetric division of a germline stem cell to produce a progenitor cell known as a cystoblast. The cystoblast then divides exactly four times to generate 16 interconnected " cystocytes" (Fig. 1A). These cystocyte cell cycles have several unusual features. All the cystocytes within a developing cyst divide synchronously, ensuring that the final cell number corresponds to a power of 2. In addition, cytokinesis remains incomplete during M phase, leaving the daughters connected by cytoplasmic bridges known as ring canals. Cystocyte cell cycles are rapid and are uncoupled from cell growth, leading to a progressive reduction in cell volume. Finally, the divisions are polar such that the cells in a completed 16-cell cyst are always interconnected in a defined pattern that presages oocyte specification.

The onset of meiosis and oocyte differentiation within completed cysts also involves complex cell cycle changes. Only one cystocyte from each cyst differentiates into an oocyte, while the remaining 15 become nurse cells that nourish the oocyte with material transported through the ring canals. However, all 16 cystocytes initially enter a characteristic long premeiotic S phase; many if not all of the cells progress into meiotic prophase as evidenced by the production of synaptonemal complex material and recombination nodules (Carpenter, 1981; Schmekel *et al.,* 1993). Over a period of time, though, only the future oocyte remains in meiosis. The other 15 cystocytes lose synaptonemal complexes and remain stalled in a poorly understood cell cycle state. These pronurse cells initiate another S phase about 4 days after cyst completion but never divide again. Instead they grow into polyploid nurse cells via a series of endocycles that preserve their interconnections (reviewed in Spradling, 1993). Early microtubule-dependent transport of molecules from the pronurse cells into the pro-oocyte prior to the onset of nurse cell endocycles is

[1] Present address: Cell Biology and Molecular Genetics, H. J. Patterson Hall, University of Maryland, College Park, MD 20742.

0012-1606/00 $35.00

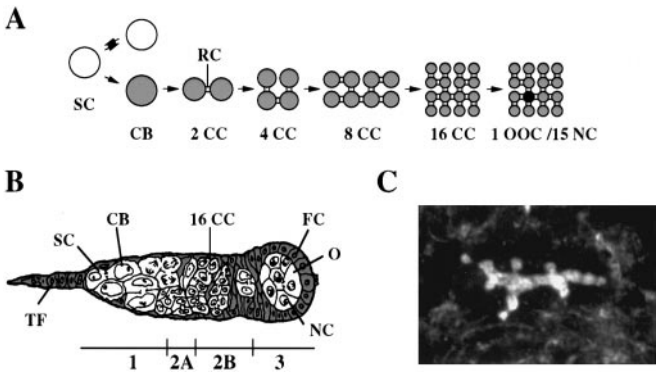

FIG. 1. Germline cyst formation in the *Drosophila* ovary. (A) The pattern of cyst connections resulting from the four synchronous cystocyte divisions (reproduced, by permission of the publisher, from de Cuevas *et al.,* 1996). (B) Schematic of a germarium, reproduced, by permission of the publisher, from King (1970). The three regions of the germarium are noted underneath. Germline cells are shown in white and include (SC) germline stem cell, (CB) cystoblast, (16 CC) 16 cell cyst, (O) oocyte, and (NC) nurse cell. The somatic cells are shown in gray and include the (FC) somatic follicle cells that envelope the developing germline cysts and the (TF) terminal filament. (C) Fusome from a 16-cell cyst stained with mAb1B1.

essential for oocyte development, but the nature of the critical molecules remains unknown (Suter and Steward, 1991; Theurkauf *et al.,* 1993).

Several genes that help specify the growth and division of early germ cells have been identified. Partial loss-of-function mutations of the G1 cyclin gene, *cyclin E,* perturb the germline cell cycles and cause one or two additional cystocytes to develop as oocytes (Lilly and Spradling, 1996). Mutations in several genes involved in meiotic recombination arrest oocyte nuclear development and disrupt oocyte patterning (Ghabrial *et al.,* 1998; Morris and Lehmann, 1999). How germline cell cycles are programmed and how they interact with other pathways to control oocyte determination and differentiation remain to be elucidated.

Another important factor in germ cell differentiation is an unusual cytoplasmic structure known as the fusome (Fig. 1C) that is present in premeiotic adult germ cells and developing cysts (reviewed in McKearin, 1997). This vesicle-rich organelle, which grows new branches after each cyst division, passes through the ring canals and physically connects all the cystocytes within a single cyst (de Cuevas *et al.,* 1997). Membrane skeletal proteins including α- and β-Spectrin, Ankyrin, and the Adducin-like product of the *hu-li tai shao* (*hts*) gene are abundant within the fusome (Lin *et al.,* 1994; de Cuevas *et al.,* 1996). During the synchronous divisions of the cystocytes, one pole from each mitotic spindle is associated with the fusome (reviewed in de Cuevas *et al.,* 1997); thus, it has long been thought that the fusome plays a role in determining the invariant pattern of cystocyte connections by orienting mitotic spindles (Telfer, 1975).

The molecular mechanisms the fusome uses to influence the cell cycle are not understood. Mutations in the genes encoding fusome components α-Spectrin or Hts eliminate the organelle almost entirely and cause characteristic changes in cystocyte behavior. Mitotic synchrony is disrupted and the number of cystocyte divisions reduced; moreover, the mutant cysts almost always fail to form an oocyte (Yue and Spradling, 1992; de Cuevas *et al.,* 1996). Mutations in the genes encoding two fusome-associated proteins, Bag-of-marbles (Bam) and Dynein heavy chain (Dhc64c), a component of the motor protein Dynein, disrupt normal fusome structure and alter the cyst division cycle (McKearin and Spradling, 1990; McKearin and Ohlstein, 1995; Gonczy *et al.,* 1997; McGrail and Hays, 1997; McKearin, 1997). Interestingly, the association of Dhc64c with the fusome is cell cycle dependent (McGrail and Hays, 1997).

Several aspects of premeiotic germ cell development appear to be widely conserved in evolution (Pepling *et al.,* 1999). Consequently, a better understanding of the relationship between cell cycle programming and germ cell behavior is likely to be of widespread significance. In eukaryotes, the sequential activation of cyclin-dependent kinases (cdks) is responsible for the ordered progression of the cell cycle (reviewed in Nurse, 1994; Nigg, 1995). Cyclins are a highly conserved family of proteins that serve as positive regulatory subunits of cdks; individual cyclins bind and activate a defined subset of cdks. In mammalian cells, Cyclin A (CycA) associates with two different cdks in distinct but overlapping phases of the cell cycle (D'Urso *et al.,* 1990; Pagano *et al.,* 1992; Rosenblatt *et al.,* 1992). CycA–Cdk2 activity peaks in S phase, whereas CycA–Cdk1 activity peaks in G2, before the onset of mitosis. Consistent with CycA being required during two points in the cell cycle, antibodies against CycA block both S-phase entry and mitosis (Swenson *et al.,* 1986; Roy *et al.,* 1991; Girard *et al.,* 1991; Pagano *et al.,* 1992; Jackson *et al.,* 1995)

In *Drosophila,* CycA has a defined role in mitosis but is not absolutely required for S phase (Lehner and O'Farrell, 1989, 1990). Specifically, in the endocycle, DNA replication proceeds in the absence of CycA (Lehner and O'Farrell, 1990; Knoblich *et al.,* 1994; Lilly and Spradling, 1996). Although it is not essential for DNA replication, there is considerable evidence that in *Drosophila,* as in vertebrates, CycA can influence the transition from G1 to S phase. For example, in developing eye discs, the expression of CycA from a heat shock promoter, or the inappropriate presence of CycA during G1 in *roughex* mutants, forces cells to exit G1 prematurely and enter S phase in the morphogenetic furrow (Thomas *et al.,* 1994, 1997; Dong *et al.,* 1997). Similarly, the overexpression of CycA drives cells from G1 into S phase in both wild-type embryos and mutant embryos lacking the G1 cyclin, Cyclin E (CycE) (Sprenger *et al.,* 1997). Thus in *Drosophila,* as in other organisms, CycA can influence the entry into both S phase and mitosis.

Mitotic cyclins are degraded by a ubiquitin-dependent proteolytic system (reviewed in Murray, 1995; Koepp *et al.*, 1999). The *UbcD1* gene encodes a type I E2 ubiquitin-conjugating enzyme with substantial homology to *Saccharomyces cerevisiae* Ubc4 (Treier *et al.*, 1992). In *Xenopus* egg extracts, the Ubc4 homolog supports Cyclin B (CycB) ubiquitination, suggesting that it may be functionally redundant with the cyclin-selective E2, E2-C (King *et al.*, 1995). In *Drosophila*, mutations in *UbcD1* result in inappropriate telomere to telomere associations in both mitosis and male meiosis (Cenci *et al.*, 1997). However, the biochemical role of UbcD1 remains undefined.

MATERIALS AND METHODS

Drosophila Strains and Culture

Flies were grown on standard corn meal/agar media at 22–25°C. The *Hs-cyclin A*, *Hs-Δcyclin A*, *Hs-cyclin B*, *Hs-Δcyclin B*, *Hs-cyclin E*, and *Df(2R)59AB* stocks were provided by Christian Lehner (Knoblich and Lehner, 1993; Knoblich *et al.*, 1994; Sigrist *et al.*, 1995). A lethal allele of *cyclin A*, $cycA^{1545}$ (Karpen and Spradling, 1992), and two female sterile alleles of *UbcD1*, $UbcD1^{1295}$ (Karpen and Spradling, 1992) and $UbcD1^{RK1859}$ (Kelly, 1993), were used. The $UbcD1^{RK1859}$stock was provided by Rick Kelly. *Df(2L)r10* was provided by J. Roote and M. Ashburner. The $bam^{\Delta 86}$ stock was a gift from Dennis McKearin (McKearin and Ohlstein, 1995). Other genes and alleles used are described on Flybase (http://flybase.bio.indiana.edu:80/.bin/fbgenq.html).

Heat Shock Protocols

For each heat shock treatment, food vials containing flies were placed in a 37°C water bath for 30 min. Heat shocks were repeated at 12-h intervals over 2 days for a total of four treatments. Between heat shocks, flies were placed at 22–25°C. After the final heat shock, flies were cultured for 4–5 days before being dissected, to allow egg chamber growth to proceed. To examine fusomes after cyclin overexpression, flies were dissected not more than 12 h after the final heat shock.

BrdU Labeling

Ovaries were dissected in Grace's medium (Gibco BRL) and incubated for 1 h in 1 μg/ml BrdU (Sigma) diluted in Grace's medium. The ovaries were then processed as described in Lilly and Spradling (1996).

Immunohistochemistry and Microscopy

Ovaries were dissected, fixed, immunologically stained, and mounted as described in Lin *et al.* (1994). Mouse monoclonal antibodies A12 and A19 against CycA and F2F4 against CycB, used at dilutions of 1:1 and 1:4, respectively, and the rabbit antibody against CycA, used at a dilution of 1:50, were provided by Patrick O'Farrell (Lehner and O'Farrell, 1990; Sprenger *et al.*, 1997). The monoclonal antibody 8B10 against *Drosophila* CycE (Richardson *et al.*, 1995) was provided by Helena Richardson and used at a dilution of 1:8. Fusomes were labeled using the monoclonal antibody 1B1 (Zaccai and Lipshitz, 1996) at a dilution of 1:5. FITC-conjugated anti-mouse and TRITC-conjugated anti-rabbit secondary antibodies were purchased from Jackson ImmunoResearch Laboratories and used at a dilution of 1:200. Ovaries were stained with DAPI and propidium iodide as described in Lilly and Spradling (1996). Stained ovaries were examined by epifluorescence and confocal microscopy on a Zeiss laser scan microscope, a Zeiss 410 confocal microscope, or a Leica TCS-NT confocal microscope. Testes were dissected from adult males 3–5 days of age and treated and examined essentially as described above.

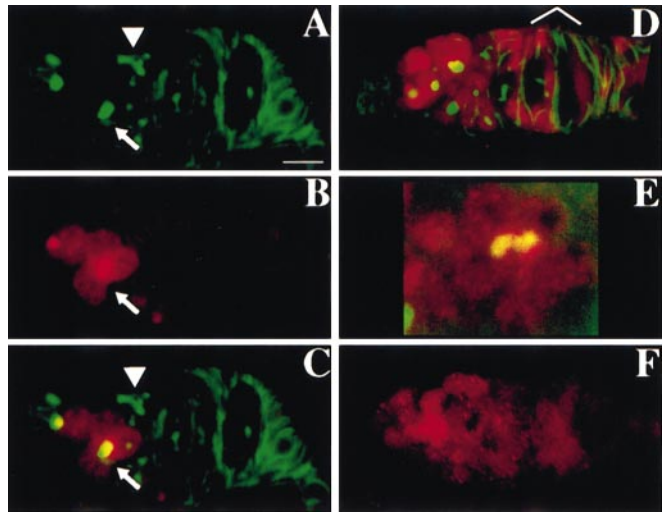

FIG. 2. CycA colocalizes with fusomes. A single confocal section from a wild-type germarium stained with (A) mAb1B1 and (B) anti-CycA. (C) The overlay of A and B reveals that CycA is concentrated on the fusome in some cysts (arrow) but is absent from the fusome in others (arrowhead). (D) A single confocal section from anti-CycA and anti-Hts double labeling demonstrates that CycA is not present in the germline in cysts in regions 2b and 3 in the germarium (bracket). (E) A confocal section of a testis double labeled with anti-CycA and mAb1B1 shows CycA at high levels on the fusome of a four-cell cyst. (F) A hts^{1} germarium stained with anti-CycA. In all images, mAb1B1 is shown in green, and anti-CycA is shown in red. Bar, 10 μm.

RESULTS

Cyclin A Associates with the Fusome

To investigate the cell cycles of early *Drosophila* germ cells, we stained wild-type germaria from adult females with specific antibodies that recognize CycA, CycB, or CycE. All three proteins were expressed in a fraction of the stem cells and growing cystocytes, as expected for mitotically cycling cells (data not shown). However, these experiments also revealed an unanticipated behavior of CycA during these developmental stages (Fig. 2). When wild-type ovaries were fixed and double labeled with antibodies against Hts (Fig. 2A) (Zaccai and Lipshitz, 1996), a marker for the fusome, and CycA (Fig. 2B) (Lehner and O'Farrell,

1990; Sprenger *et al.*, 1997), we observed that CycA was concentrated on the fusome in some, but not all, germline cysts (Fig. 2C). For example, only one of the two round fusomes which mark the two stem cells at the anterior end of the germarium displays fusome-associated CycA (Fig. 2C). Likewise, the fusome in one growing cyst is labeled by anti-CycA antibody (arrow) while the fusome from another similar cyst (arrowhead) remains unlabeled. CycA was associated only with the fusomes of stem cells and growing cysts, and never with fusomes in 16-cell cysts in regions 2 and 3 (Fig. 2D). Thus, CycA is never concentrated on the fusome in ovarian germline cysts that have completed their mitotic program. Similar observations were made using three independent antibodies generated against *Drosophila* CycA protein. Neither CycB nor CycE was enriched on the fusome (data not shown). CycA, but not the other cyclins, transiently associated with the fusome during the mitotic divisions in male germline cysts as well (Fig. 2E).

The aggregation and enrichment of CycA at the site of the fusome might be the result of a specific interaction between CycA and a fusome component. Alternatively, both the fusome and the CycA aggregates might be independently colocalized within the same cellular compartment by a common process. To distinguish between these possibilities, we examined whether cytoplasmic CycA aggregates are observed in the ovaries of *hts* mutant females. These females lack fusomes but are able to form small, abnormal cysts. We found that CycA fails to concentrate within any part of the cytoplasm of *hts*^{1} mutant germ cells (Fig. 2F). Since an intact fusome is required for CycA aggregation, it is likely that CycA associates specifically with the fusome during a portion of the cell cycle in stem cells and forming cysts.

The CycA–Fusome Association Occurs during G2 and Prophase

To determine when CycA associates with the fusome, we first estimated the fraction of the early germ cells at various stages with fusome-associated CycA. Twenty-three germaria were double stained to visualize Hts and CycA and examined with a confocal microscope, and positive fusomes were recorded. About 40% of stem cells and 15% of forming cysts in region 1 displayed fusome-associated CycA (Table 1).

The appearance of fusomes changes in a regular way during stem cell and cystocyte divisions (Deng and Lin, 1997; de Cuevas and Spradling, 1998). During G1 and S phase, the stem cell fusome elongates and fuses with the growing "plug" of new fusome material that is located in the transient ring canal between stem cell and daughter cystoblast. This ring canal pinches closed in G2 phase, so that late G2 stem cells contain fusomes that are round (de Cuevas and Spradling, 1998). In stem cells, CycA associated only with round fusomes (Table 1). During cystocyte divisions, cells in G1 and early S phase can be recognized because newly growing fusome "plugs" have not yet fused to the parent fusome (M. de Cuevas and A. Spradling, unpublished). In contrast, cells in late S or G2 display a single fusome structure with all branches connected. CycA associated with fused fusomes in growing cysts. Thus, the association of CycA with the fusome appears to take place primarily during the G2 phase in both stem cells and cystocytes.

TABLE 1
Association of Cyclin A with Fusomes of Specific Morphology

Fusome morphology	Cyclin A+	Cyclin A−
Round (mid-G2–M stem cell)	22 (40%)[a]	15
Elongated (G1–early G2 stem cell)	0	20
2-, 4-, 8-cell cyst fusome	12 (15%)[b]	90[c]
16-cell cyst fusome	0	160[c]

[a] 22 CycA+ stem cells/57 total stem cells = 40%.
[b] 12 CycA+ mitotic cysts (i.e., those with a 2-, 4-, or 8-cell cyst fusome)/90 total mitotic cysts = 15%.
[c] The estimated number of cysts of the indicated stages present in the 23 germaria examined.

We confirmed these conclusions by examining CycA–fusome associations in cells in which specific cell cycle stages had been identified using molecular markers (data not shown). After ovaries were pulse labeled with the nucleotide analog BrdU to identify S phase, fusome-associated CycA was found almost exclusively in unlabeled cells. However, CycA labeled the fusome weakly in a few cysts with late S-phase patterns of BrdU incorporation. In ovaries that were double labeled with antibodies against CycE or CycB, CycA was never observed on the fusome in cysts that were positive for the G1 cyclin, CycE. In contrast, the cytoplasm of cysts was positive for the G2 cyclin, CycB, when CycA was concentrated on the fusome. These results confirm that CycA is on the fusome in G2 but not in G1 and early S phase.

Figure 3 summarizes the interaction of CycA with the fusome during the cell cycle. In G1 and most of S phase, there is limited CycA accumulation and it is not fusome associated (Fig. 3A). Beginning in late S or G2, CycA begins to associate with the fusome and accumulates there at high levels just prior to mitosis. In prophase cells with partially condensed chromosomes, CycA levels are high on the fusome, while lower amounts can be seen throughout the rest of cyst (Fig. 3B). As mitosis progresses, CycA levels throughout the cyst rise to the same high levels that were previously observed only on the fusome (Fig. 3C). Finally, at approximately the metaphase to anaphase transition, CycA is degraded throughout the cyst (Fig. 3D).

CycA Overexpression Forces Ovarian Germline Cysts through a Fifth Mitotic Division

The periodic association of CycA with the fusome suggests that the precise spatial and temporal regulation of cell

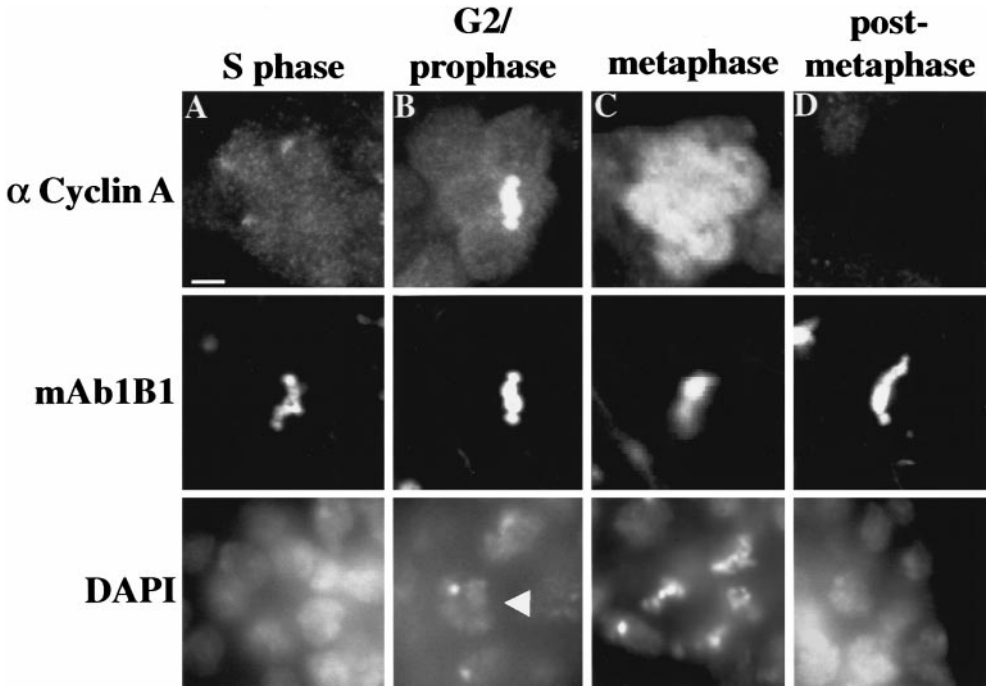

FIG. 3. The distribution of CycA during the cystocyte divisions. (A–D) Ovaries were stained with antibodies raised against *Drosophila* CycA and the monoclonal antibody mAb1B1 to highlight fusomes and follicle cell membranes. DNA is labeled with DAPI. A single cyst in the indicated cell cycle stage is shown in each part. (A) S phase. The distribution of CycA during S phase was determined by performing BrdU incorporation in concert with anti-CycA antibody staining (data not shown). (B) G2/prophase. The DNA has begun to condense (arrowhead). (C) Metaphase. (D) Postmetaphase. For all images, not all cells within the dividing cyst are in the plane of focus. Bar, 5 μm.

cycle regulatory proteins may be required to direct the specialized cystocyte division cycle. To investigate its role in cyst formation, we overexpressed CycA in adult flies using two transgenes (Sigrist *et al.*, 1995) that are under the control of the heat-inducible hsp70 promoter. Although these transgenes produce similar, high amounts of CycA protein in the ovary following heat shock treatment (data not shown), the two proteins they encode differ significantly in their relative stabilities. The first transgene, *Hs-cyclin A*, encodes full-length CycA; the second transgene, *Hs-*Δ*cyclin A*, encodes a truncated ΔCycA whose N terminus, including the destruction box, is deleted. The destruction box is a 9-amino-acid motif that targets CycA and other mitotic cyclins for rapid ubiquitin-dependent proteolysis late in mitosis (Glotzer *et al.*, 1991). The deletion of the destruction box significantly increases the stability of the ΔCycA protein relative to CycA (Sigrist *et al.*, 1995; M. Lilly and A. Spradling, unpublished). Although it contains an N-terminal deletion, the *Hs-*Δ*cyclin A* construct encodes a functional protein as assayed by its ability to rescue partially the embryonic phenotype of a null mutation of the *cyclin A* gene (Sigrist *et al.*, 1995).

When CycA is overexpressed in adult females from these transgenes, germline cysts with an aberrant number of cells are produced. Five days after heat-shock treatment, germline cysts containing 32 instead of 16 cells were seen in 17% of egg chambers from *Hs-*Δ*cyclin A* ovaries and 3% of egg chambers from *Hs-cyclin A* ovaries (Figs. 4A and 4B, Table 2). We also observed a few cysts with an intermediate number of cells, between 16 and 32, but such cysts were extremely rare in both *Hs-cyclin A* and *Hs-*Δ*cyclin A* ovaries. These observations suggest that the overexpression of CycA can force cysts through one extra round of division. Moreover, the fact that almost all cysts contained either 16 or 32 cells, and not an intermediate number, suggests that the decision to divide a fifth time is an all-or-none event, like the earlier cystocyte cycles.

To confirm that the egg chambers with 32 cells contain a single large cyst, and not two 16-cell cysts that have been packaged by a single layer of follicle cells, we examined the aberrant egg chambers more closely. The 32-cell cysts produced after CycA overexpression differentiate a single oocyte nucleus (germinal vesicle) and therefore contain 31 nurse cells and 1 oocyte (Fig. 4B). Furthermore, the oocytes in the 32-cell cysts contain five ring canals, consistent with an extra mitotic division (data not shown). Thus, the extra mitosis and resulting increase in cystocyte number appears to take place in a manner similar to early cystocyte divisions.

In addition to our experiments with CycA, we also

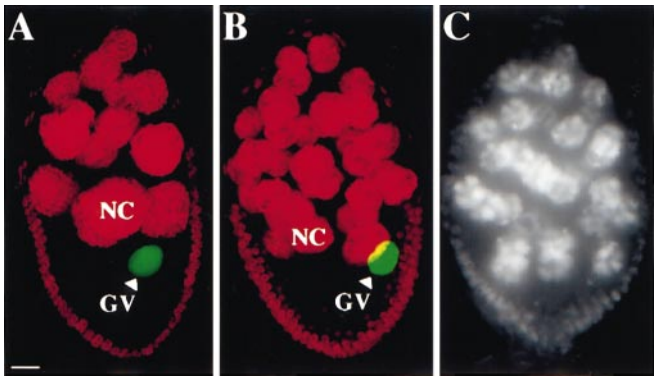

FIG. 4. The overexpression of CycA results in an extra cyst division. Egg chambers from (A) wild-type and (B) *Hs-Δcyclin A* females were stained with propidium iodide to label nuclei and with a low concentration of anti-CycE to highlight the germinal vesicle (GV). Note that the *Hs-Δcyclin A* egg chamber contains only a single GV. (C) A DAPI-stained egg chamber containing a 32-cell germline cyst from a *Hs-Δcyclin B* female. In all images some nurse cell nuclei lie outside the plane of focus.

examined the effects of overexpressing CycB or CycE. All cyclin transgenes are under the control of the heat-inducible hsp70 promoter. When the destruction-box-deleted G2 cyclin, ΔCycB, was overexpressed in adult flies, egg chambers containing 32-cell cysts were produced, albeit at a rate approximately three times lower than in the *Hs-Δcyclin A* flies (Fig. 4C, Table 2). In contrast the overexpression of the G1 cyclin, CycE, did not result in the production of egg chambers with 32 cells at a measurable rate (Table 2). Although CycE does not contain a destruction box, it does have multiple PEST domains that are predicted to render the protein unstable (Richardson *et al.*, 1993). The inability of CycE overexpression to induce an extra cyst division may be due to the instability of CycE relative to destruction-box-deleted G2 cyclins or to an intrinsic difference between CycE and the G2 cyclins.

Mutations in an E2 Ubiquitin-Conjugating Enzyme Phenocopy CycA Overexpression

If the number of cyst divisions can be influenced by the inappropriate perdurance of cyclin activity, then mutants that alter cyclin stability may affect the number of cells per cyst. Consistent with this proposal, we determined that mutations in the *UbcD1* gene, which encodes an E2 ubiquitin-conjugating enzyme, can cause cysts to divide a fifth time (Fig. 5A). Approximately 22% of egg chambers from $UbcD1^{1295}/UbcD1^{RK1859}$ *trans*-heterozygous females and 5% of egg chambers from females homozygous for the weak *UbcD1* mutation, $UbcD1^{1295}$, contained 32-cell cysts (Fig. 5B). As was observed after CycA overexpression, the vast majority of egg chambers contained either 16 or 32 germline cells and differentiated a single oocyte; cysts with an intermediate number of cells were rare. The $UbcD1^{RK1859}$ mutation is semilethal and therefore homozygotes could not easily be examined.

We then asked whether mutations or deficiencies of the cyclin genes could dominantly suppress the extra cyst divisions observed in *UbcD1* mutants (Fig. 6). We found that when the dosage of the mitotic cyclins was reduced, the *UbcD1* 32-cell phenotype was suppressed. A single copy of a *cyclin A* lethal allele, $cycA^{1545}$, reduced the production of 32-cell cysts in $UbcD1^{1295}/UbcD1^{RK1859}$ females from 25 to <6%, an approximately fourfold reduction. A deletion of the *cyclin B* gene had a similar but reduced effect; one copy of the *cyclin B* deficiency *Df(2R)59AB* lowered the number of 32-cell cysts by threefold. In contrast, *Df(2L)r10,* which deletes the *cyclin E* gene, had relatively little effect on the production of 32-cell cysts in the *UbcD1* mutant background. These data support the idea that the inappropriate perdurance of cyclin activity causes the extra cyst division observed in *UbcD1* mutants.

Extra Cyst Divisions Are Accompanied by the Growth of the Fusome and Are Dependent on the bam Gene Product

How is the number of cyst divisions limited to precisely four in wild-type flies? The association of CycA with the fusome, as well as the observation that CycA overexpression can induce an extra cyst division, suggests that the temporal and spatial regulation of cell cycle regulatory proteins in 16-cell cysts may be an important part of the limiting mechanism. To test this idea, we wanted to determine if CycA acts through the normal cyst division machinery to induce an extra division or if it bypasses it. The protein expressed by the gene *bag of marbles* (*bam*) is required for cyst formation and is found on fusomes and in the cytoplasm of dividing cystocytes (McKearin and Spradling, 1990; McKearin and Ohlstein, 1995). Furthermore, the cytoplasmic form of Bam disappears soon after the final cyst division. These observations have led to the suggestion that Bam may play a role in counting the cyst divisions. Thus, we wanted to determine if the ΔCycA overexpression phenotype is sensitive to levels of Bam in the cyst. We found that a

TABLE 2
32-Cell Cyst Induction (%) 5 Days after Cyclin Overexpression

Genotype	32-cell cysts (%)[a]
Wild type	0
Hs-ΔcyclinA	17 ± 1.9
Hs-cyclinA	2.6 ± 0.75
Hs-ΔcyclinB	5.9 ± 1.5
Hs-cyclinE	0.4 ± 0.11

[a] At least 900 egg chambers, representing more than 10 ovaries, were examined for each genotype.

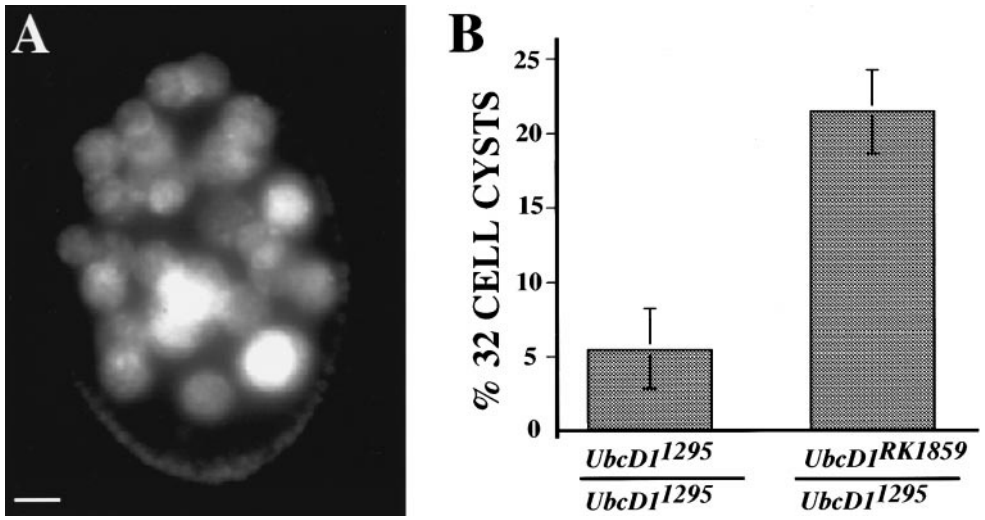

FIG. 5. *UbcD1* females produce egg chambers with 32 germline cells. (A) An egg chamber from a $UbcD1^{1295}/UbcD1^{RK1859}$ female with 31 nurse cells and 1 oocyte. Bar, 10 μm. (B) The percentage of egg chambers with 32 germline cells observed in *UbcD1* mutant females.

bam null allele, $bam^{\Delta 86}$, can dominantly suppress ΔCycA overexpression; 2.6% of the egg chambers from *Hs-*Δ*cyclin A* females in which the dose of *bam* was reduced by one copy contained 32-cell cysts after heat-shock treatment in comparison to 13% observed in sibling *Hs-*Δ*cyclin A* females that contained two functional copies of *bam* ($n > 850$). This represents a fivefold reduction in the production of 32-cell cysts. These data suggest that CycA acts through the normal cyst division regulatory machinery to trigger a fifth round of mitosis.

Does the extra round of cystocyte division result in the growth of the fusome as is observed during the first four mitotic cyst divisions? In $UbcD1^{1295}/UbcD1^{RK1859}$ females, in which up to 25% of germline cysts contain 32 cells, we observed large fusomes with an increased number of branches relative to wild-type cysts (Fig. 7). Large fusomes were also observed in *Hs-*Δ*cyclin A* females after heat-shock treatment (data not shown). Abnormally large fusomes were never observed in wild type females or in *Hs-*Δ*cyclin A* females that had not been heat shocked. Thus, the fifth cyst division in multiple genetic backgrounds drives the corresponding growth of the fusome.

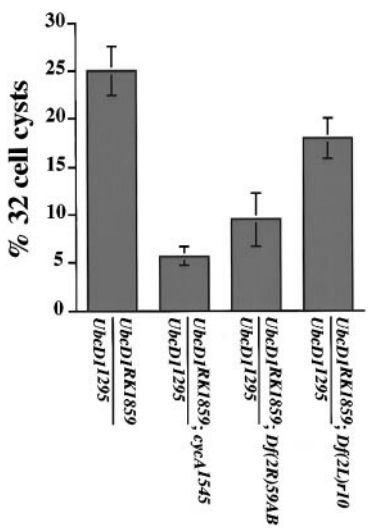

FIG. 6. The *UbcD1* 32-cell cyst phenotype is suppressed by mutations in *cyclin A* and *cyclin B*. The percentage of 32-cell cysts present in the ovary are compared between the following four genotypes: $UbcD1^{1295}/UbcD1^{RK1859}$, $UbcD1^{1295}/UbcD1^{RK1859}$; $cycA^{01545}/+$, $UbcD1^{1295}/UbcD1^{RK1859}$; *Df(2R)59AB/+*, and $UbcD1^{1295}/UbcD1^{RK1859}$; *Df(2L) r10/+*.

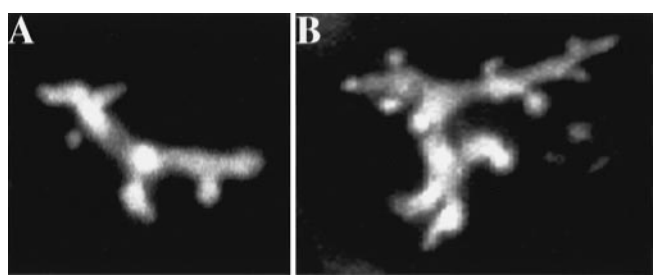

FIG. 7. *UbcD1* females have highly branched fusomes. The monoclonal antibody mAb1B1 was used to label fusomes in (A) wild-type or (B) $UbcD1^{1295}/UbcD1^{RK1859}$ germaria. Note that the fusome from the $UbcD1^{1295}/UbcD1^{RK1859}$ female is larger and more branched than wild type.

DISCUSSION

Association of CycA with the Fusome May Synchronize the Germline Cyst Divisions

The development of the *Drosophila* germ line uses a succession of different cell cycles to help effect dramatic changes in cell size and structure. Early germ cells are single, large round cells, while at the onset of meiosis, they are smaller and interconnected within groups of 16 by ring canals. The molecular mechanisms that reprogram germ cell cycles are only beginning to be understood. Our studies have revealed several new aspects of these processes.

The fusome is likely to program at least one important aspect of germ cell cycles, the synchronization of the cystocyte divisions. First, the fusome occupies a key location within growing cysts for mediating synchrony. By the onset of mitosis at each round of cyst division, the fusome has completed its cycle of growth and new segments have fused into a single continuous structure. The branches of the G2 fusome pass uninterrupted through the ring canals that interconnect the cystocytes, providing a single internal interface between all cyst cells. Second, synchronous cell cycles occur only while the fusome is present. Cell–cell junctions alone are insufficient to guarantee synchrony, since ring canals remain present in older cysts when the cells cycle asynchronously. Finally, genetic data support a role for the fusome in cystocyte synchrony. Mutations that disrupt the fusome disrupt synchrony, as indicated by the production of cysts that no longer contain 2^n cells (Lin *et al.*, 1994; de Cuevas *et al.*, 1996; reviewed in de Cuevas *et al.*, 1997).

Our studies suggest that the fusome effects cystoctye synchrony through an interaction with CycA. CycA associates with the fusome only in synchronously cycling cystocytes, and not in 16-cell cysts. CycA–fusome association begins in G2 when CycA levels on the fusome rise above those observed in the rest of the cyst. By early prophase CycA levels on the fusome are much higher than in the surrounding cytoplasm. As mitosis progresses CycA is seen at high levels throughout the cyst before being destroyed at approximately the metaphase-to-anaphase transition. The accumulation of CycA on the fusome prior to the activation of CycA/Cdk1 at the G2/M transition suggests that CycA association with the fusome synchronizes the entry of all the interconnected cystocytes within a cyst into mitosis.

How might CycA effect mitotic synchrony through its association with the fusome? The fusome most likely acts in some way to spatially equalize the activation of CycA/Cdk1 activity in all cystocytes during late G2. In yeast, Cyc/Cdk activation is controlled by changes in the levels of stimulatory and inhibitory phosphorylation at specific target sites. The phosphatases and kinases carrying out these changes are themselves subject to control by cdk-mediated phosphorylation to generate a strong positive feedback loop (King *et al.*, 1994). We propose that enzymes responsible for CycA/Cdk1 activation are present in the fusome and are subject to similar positive feedback control. Activation of CycA/Cdk1 activity in any portion of the fusome might spread to adjacent molecules, causing a wave of activation to spread rapidly throughout the fusome. The ability of the fusome to propagate an active state of CycA/Cdk1 would synchronize the mitotic entry of all cystocytes that retained an intact fusome.

This model makes several testable predictions. Cdk1 and regulatory molecules should be found within the fusome. CycA/Cdk1 activation should occur in a transient wave that spreads along the fusome. Breaking the fusome should divide the cyst into independently cycling cystocyte subgroups, each joined by an intact fusome segment. Cyclin A has been observed previously to associate with the fusome in the postmitotic 16-cell cysts of *Drosophila* males (Eberhart *et al.*, 1996). Our observation that CycA also associates with the fusome during male cystocyte divisions (Fig. 2E) suggests that the proposed mechanism may also operate during male cyst formation.

Specifying the Number of Cystocyte Divisions

Germline cysts in different organisms contain a species-specific, programmed number of cystocytes. Thus, while *Drosophila* male and female germline cysts contain 16 cells, cysts in Lepidoptera such as *Bombyx mori* consist of only 8 cells, while cysts in many Hymenoptera such as *Habrobracon juglandis* are made up of 32 cells (see Büning, 1994). In each case, cystocyte divisions take place synchronously, so the differences in cyst cell number result from a different number of rounds of division. Currently, little is known about what determines the number of rounds of synchronous cystocyte divisions.

We found that misexpressing two G2 cyclins increases the number of cystocytes in *Drosophila* ovarian cysts. Several observations indicate that the increase is due to the induction of one additional round of relatively normal, synchronous cystocyte division. Cysts that undergo additional mitoses contain exactly twice the normal number of cystocytes, 32 instead of 16. The 32-cell cysts contain fusomes with additional branches as expected for an additional normal division, and the oocyte contains five instead of four ring canals. The 32-cell cysts still differentiate a single oocyte, indicating that all the cells remain functionally interconnected and become normally polarized. Finally, as in normal divisions, Bam protein is required for the additional division since a *bam* null mutation acts as a dominant suppressor of the 32-cell-cyst phenotype.

There are at least two possible ways that G2 cyclin misexpression might produce cysts with more cells. In the first model, we propose that cyst cell number is determined by cyst size. The number of cystocytes per cyst would simply depend on the number of divisions that occur during the time required for the cyst to grow to an appropriate size, as determined by intercellular signals. G2 cyclin misexpression would shorten cystocyte cell cycles so that an addi-

tional cycle occurs by the time the stop signal is received. Either G1 or G2 or both may be affected. Inappropriate presence of CycA during the G1 phase has been shown to cause premature entry into S phase in embryos (Sprenger *et al.*, 1997) and developing eye discs (Thomas *et al.*, 1994, 1997; Dong *et al.*, 1997). In addition, high levels of both CycA and CycB might shorten the G2 phase of the cystocyte cycle. The inability of CycE overexpression to induce an extra division may be more consistent with action of CycA and CycB in G2.

Alternatively, rather than accelerating cell cycles, inappropriately high levels of G2 cyclins in 16-cell cysts after the completion of premeiotic S phase may interfere with mechanisms that normally shut off further cystocyte cell cycles after four rounds have been completed. This second model proposes that the down-regulation of G2 cyclin activity in 16-cell cysts limits the number of mitotic divisions to four. If G2 cyclin activity remains inappropriately high after premeiotic S phase, 16-cell cysts would fail to exit the mitotic cycle and would instead execute an additional division. In this model the inability of CycE overexpression to promote a fifth cyst division is due to the fact that CycE is a G1 and not a G2 cyclin. It is possible that both mechanisms of producing 32-cell cysts may have occurred in our experiments.

Both models assume that cyclin misexpression acts directly on the developing germ cells, rather than indirectly through changes in nearby somatic cells. Several observations suggested that this was probably a safe assumption. The somatic cells that contact growing cystocytes are not themselves mitotically active, and BrdU incorporation was not detected in these cells following cyclin misexpression. Moreover, increased cyst cell number depended on normal levels of Bam, a gene expressed only in the germline. Nonetheless, it would be worthwhile to target cyclin misexpression specifically to germ cells to further investigate this issue.

Two other genes that alter cyst cell number may act in a related manner. Females that carry a hypomorphic allele of *cyclin E,* or that are mutant for *encore,* frequently contain only 8 or 32 cells per cyst, respectively (Lilly and Spradling, 1996; Hawkins *et al.*, 1996). Reduced CycE levels may slow the cystocyte cell cycles. Encore is thought to function in concert with other gene products in translationally regulating localized germline mRNAs (Hawkins *et al.*, 1997). Altered target gene expression in *encore* mutant females may accelerate the cystocyte cell cycles or interfere with the normal mechanism that shuts off these cycles.

Ubiquitin-Dependent Proteolysis Influences the Number of Germline Cyst Divisions

UbcD1 appears to influence the degradation of a wide array of proteins, including those involved in chromosome segregation (Cenci *et al.*, 1997; Tang *et al.*, 1997). However, the effects we observed on germline cyst formation may derive from a direct action on cyclin turnover. In *Xenopus* egg extracts the UbcD1 homolog, Ubc4, supports the ubiquitination of CycB (King *et al.*, 1995; Yu *et al.*, 1996). Destruction-box-deleted ΔCycA and ΔCycB are considerably more effective at forcing cysts though an extra cyst division than the corresponding wild-type proteins. Moreover, mutations in *cyclin A* and *cyclin B* dominantly suppress the extra cyst division observed in *UbcD1* mutants. Thus, the stabilization of CycA and CycB in *UbcD1* mutants is likely to play a role in triggering the fifth mitotic division. Like misexpressed G2 cyclins, the lower level of proteolytic machinery in *UbcD1* mutant females might accelerate the cystocyte cell cycle or result in the inappropriate activity of CycA/Cdk1 in 16-cell cysts. However, further study will be required to prove conclusively that G2 cyclin misexpression and *UbcD1* mutation act within germ cells in the postulated manner.

ACKNOWLEDGMENTS

We thank Patrick O'Farrell for providing anti-CycA and anti-CycB antibodies and Helena Richardson for providing anti-CycE antibody. We are grateful to Christian Lehner for sending the Hs-cyclin A, Hs-Δcyclin A, Hs-cyclin B, Hs-Δcyclin B, and Df(2R)59AB strains and to Dennis McKearin for the strain BamΔ86. We thank Barbara Thomas, Nicole Grieder, and Erika Matunis for comments on the manuscript. This work was supported in part by NIH Grant GM27875. A.C.S. is an Investigator of the Howard Hughes Medical Institute.

REFERENCES

Büning, J. (1994). "The Insect Ovary: Ultrastructure, Previtellogenic Growth and Evolution." Chapman & Hall, New York.

Carpenter, A. (1981). EM autoradiographic evidence that DNA synthesis occurs at recombination nodules during meiosis. *Chromosoma* **83,** 59–80.

Cenci, G., Rawson, R. B., Belloni, G., Castrillon, D. H., Tudor, M., Petrucci, R., Goldberg, M. L., Wasserman, S. A., and Gatti, M. (1997). UbcD1, a *Drosophila* ubiquitin-conjugating enzyme required for proper telomere behavior. *Genes Dev.* **11,** 863–875.

D'Urso, F., Marraccino, R. L., Marshak, D. R., and Roberts, J. M. (1990). Cell cycle control of DNA replication by a homologue from human cells of the p34cdc2 protein kinase. *Science* **250,** 786–791.

de Cuevas, M., Lee, J. K., and Spradling, A. C. (1996). α-spectrin is required for germline cell division and differentiation in the *Drosophila* ovary. *Development* **122,** 3959–3968.

de Cuevas, M., Lilly, M. A., and Spradling, A. C. (1997). Germline cyst formation in *Drosophila. Annu. Rev. Genet.* **31,** 405–428.

de Cuevas, M., and Spradling, A. C. (1998). Morphogenesis of the *Drosophila* fusome and its implications for oocyte specification. *Development* **125,** 2781–2789.

Deng, W., and Lin, H. (1997). Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in *Drosophila. Dev. Biol.* **189,** 79–94.

Dong, X., Zavitz, K. H., Thomas, B. J., Michael, L., Campbell, S., and Zipursky, S. L. (1997). Control of G_1 in the developing *Drosophila* eye: *rca1* regulates Cyclin A. *Genes Dev.* **11,** 94–105.

Eberhart, C. G., Maines, J. Z., and Wasserman, S. A. (1996). Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. *Nature* **381,** 783–785.

Ghabrial, A., Ray, R. P., and Schüpbach, T. (1998). *okra* and *spindle-B* encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in *Drosophila* oogenesis. *Genes Dev.* **12,** 2711–2723.

Girard, F., Strausfeld, H., Fernandez, A., and Lamb, H. J. (1991). Cyclin A is required for the onset of DNA replication in mammalian fibroblasts. *Cell* **67,** 1169–1179.

Glotzer, M., Murray, A. W., and Kirschner, M. W. (1991). Cyclin is degraded by the ubiquitin pathway. *Nature* **349,** 132–138.

Gonczy, P., Matunis, E., and DiNardo, S. (1997). *bag-of-marbles* and *benign gonial cell neoplasm* act in the germline to restrict proliferation during *Drosophila* spermatogenesis. *Development* **124,** 4361–4371.

Hawkins, N. C., Thorpe, J., and Schüpbach, T. (1996). *encore*, a gene required for the regulation of germ line mitosis and oocyte differentiation during *Drosophila* oogenesis. *Development* **122,** 281–290.

Hawkins, N. C., Van Buskirk, C., Grossniklaus, U., and Schüpbach, T. (1997). Post-transcriptional regulation of *gurken* by *encore* is required for axis determination in *Drosophila*. *Development* **124,** 4801–4810.

Jackson, R. K., Chevalier, S., Phillippe, M., and Kirschner, M. W. (1995). Early events in DNA replication require cyclin E and are blocked by $p21^{cip1}$. *J. Cell Biol.* **130,** 755–769.

Karpen, G. H., and Spradling, A. C. (1992). Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single P element insertional mutagenesis. *Genetics* **132,** 737–753.

Kelly, R. L. (1993). Initial organization of the *Drosophila* dorsoventral axis depends on an RNA-binding protein encoded by the *squid* gene. *Genes Dev.* **189,** 79–94.

King, R. C. (1970). "Ovarian Development in *Drosophila melanogaster.*" Academic Press, New York.

King, R. W., Jackson, P. K., and Kirschner, M. W. (1994). Mitosis in transition. *Cell* **79,** 563–571.

King, R. W., Peters, J. M., Tugendreich, S., Rolfe, M., Hieter, P., and Kirschner, M. W. (1995). A 20S complex containing cdc27 and cdc16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin-B. *Cell* **81,** 279–288.

Knoblich, J. A., and Lehner, C. F. (1993). Synergistic action of *Drosophila* cyclins A and B during the G_2–M transition. *EMBO J.* **12,** 65–74.

Knoblich, J. A., Sauer, K., Jones, L., Richardson, H., Saint, R., and Lehner, C. F. (1994). Cyclin E controls S phase progression and its down regulation during *Drosophila* embryogenesis is required for the arrest of cell proliferation. *Cell* **77,** 107–120.

Koepp, D. M., Harper, J. W., and Elledge, S. J. (1999). How the cyclin became a cyclin: Regulated proteolysis in the cell cycle. *Cell* **97,** 431–434.

Lehner, C. F., and O'Farrell, P. H. (1989). Expression and function of *Drosophila* Cyclin A during embryonic cell cycle progression. *Cell* **56,** 957–968.

Lehner, C. F., and O'Farrell, P. H. (1990). The roles of *Drosophila* Cyclins A and B in mitotic control. *Cell* **61,** 535–547.

Lilly, M. A., and Spradling, A. C. (1996). The *Drosophila* endocycle is controlled by Cyclin E and lacks a checkpoint ensuring S phase completion. *Genes Dev.* **10,** 2514–2526.

Lin, H., Yue, L., and Spradling, A. C. (1994). The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development* **120,** 947–956.

McGrail, M., and Hays, T. S. (1997). The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in *Drosophila*. *Development* **124,** 2409–2419.

McKearin, D. (1997). The *Drosophila* fusome, organelle biogenesis and germ cell differentiation: If you build it .. . . *BioEssays* **19,** 147–152.

McKearin, D., and Ohlstein, B. (1995). A role for the Bag-of-marbles protein in the differentiation of cystoblasts from germline stem cells. *Development* **121,** 2937–2947.

McKearin, D., and Spradling, A. C. (1990). *Bag of marbles:* A gene required for the initiation of both male and female gametogenesis in *Drosophila*. *Genes Dev.* **4,** 2242–2251.

Morris, J., and Lehmann, R. (1999). *Drosophila* oogenesis: Versatile *spn* doctors. *Curr. Biol.* **9,** R55–R58.

Murray, A. (1995). Cyclin ubiquitination: The destructive end of mitosis. *Cell* **81,** 149–152.

Nigg, E. A. (1995). Cyclin-dependent protein kinases: Key regulators of the eukaryotic cell cycle. *BioEssays* **17,** 471–480.

Nurse, P. (1994). Ordering S phase and M phase in the cell cycle. *Cell* **79,** 547–550.

Pagano, M., Pepperkok, R., Lukas, J., Baldin, V., Ansorge, W., Bartek, J., and Draetta, G. (1992). Cyclin A is required at two points in the human cell cycle. *EMBO J.* **11,** 961–971.

Pepling, M. E., de Cuevas, M., and Spradling, A. C. (1999). Germline cysts: A conserved phase of germ cell development? *Trends Cell Biol.* **9,** 257–262.

Richardson, H., O'Keefe, L. V., Marty, T., and Saint, R. (1995). Ectopic cyclin E expression induces premature entry into S phase and disrupts pattern formation in the *Drosophila* eye imaginal disc. *Development* **121,** 3371–3379.

Richardson, H. E., O'Keefe, L. V., Reed, S. I., and Saint, R. (1993). A *Drosophila* G_1-specific cyclin E homolog exhibits different modes of expression during embryogenesis. *Development* **119,** 673–690.

Rosenblatt, J., Gu, Y., and Morgan, D. O. (1992). Human cyclin-dependent kinase 2 is activated during the S and G2 phases of the cell cycle and associates with cyclin A. *Proc. Natl. Sci. USA* **89,** 2824–2848.

Roy, L. M., Swenson, K. I., Walker, D. H., Gabrielli, B. G., Li, R. S., Piwnica-Worms, H., and Maller, J. L. (1991). Activation of p34cdc2 kinase by Cyclin-A. *J. Cell Biol.* **113,** 507–514.

Schmekel, K., Wahrman, J., and Daneholt, B. (1993). Solitary and synaptonemal complex-associated recombination nodules in pro-nurse cells during oogenesis in *Drosophila melanogaster*. *Chromosoma* **102,** 396–402.

Sigrist, S., Jacobs, H., Stratmann, R., and Lehner, C. F. (1995). Exit from mitosis is regulated by *Drosophila fizzy* and the sequential destruction of cyclins A, B, and B3. *EMBO J.* **14,** 4827–4838.

Spradling, A. (1993). Germline cysts: Communes that work. *Cell* **72,** 649–651.

Sprenger, F., Yakubovich, N., and O'Farrell, P. H. (1997). S-phase function of *Drosophila* cyclin A and its downregulation in G1 phase. *Curr. Biol.* **7,** 488–499.

Suter, B., and Steward, R. (1991). Requirement for phosphorylation and localization of the Bicaudal-D protein in Drosophila oocyte differentiation. *Cell* **67,** 917–926.

Swenson, K. I., Farrell, K. M., and Ruderman, J. V. (1986). The clam embryo protein cyclin-A induces entry into M-phase and

the resumption of meiosis in Xenopus oocytes. *Cell* **47,** 861–870.

Tang, A. H., Neufeld, T. P., Kwan, E., and Rubin, G. M. (1997). PHYL acts to down-regulate TTK88, a transcriptional repressor of neuronal cell fates, by a SINA-dependent mechanism. *Cell* **90,** 459–467.

Telfer, W. H. (1975). Development and physiology of the oocyte–nurse cell syncytium. *Adv. Insect Physiol.* **11,** 223–319.

Theurkauf, W. E., Alterts, B. M., Jan, Y. N., and Jongens, T. A. (1993). A central role for microtubules in the differentiation of *Drosophila* oocytes. *Development* **118,** 1169–1180.

Thomas, B. J., Gunning, D. A., Cho, J., and Zipursky, S. L. (1994). Cell cycle progression in the developing *Drosophila* eye: *roughex* encodes a novel protein required for the establishment of G1. *Cell* **77,** 1003–1014.

Thomas, B. J., Zavitz, K. H., Dong, X., Lane, M. E., Weigmann, K., Finley, R. L., Brent, R., Lehner, C. F., and Zipursky, S. L. (1997). *roughex* down-regulates G_2 cyclins in G_1. *Genes Dev.* **11,** 1289–1298.

Treier, M., Seufert, W., and Jentsch, S. (1992). *Drosophila UbcD1* encodes a highly conserved ubiquitin-conjugating enzyme involved in selective protein degradation. *EMBO J.* **11,** 367–372.

Yu, H. T., King, R. W., Peters, J. M., and Kirschner, M. W. (1996). Identification of a novel ubiquitin-conjugating enzyme involved in mitotic cyclin degradation. *Curr. Biol.* **6,** 455–466.

Yue, L., and Spradling, A. C. (1992). *hu-li-tai shao,* a gene required for ring canal formation during *Drosophila* oogenesis, encodes a homolog of adducin. *Genes Dev.* **6,** 2443–2545.

Zaccai, M., and Lipshitz, H. D. (1996). Differential distributions of two adducin-like protein isoforms in the *Drosophila* ovary and early embryo. *Zygote* **4,** 159–166.

Received for publication September 20, 1999
Revised November 8, 1999
Accepted November 8, 1999